OFFICIAL PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Boyse et al.

Serial No.: 08/442,277

Group Art Unit: 1804

Filed: May 16, 1995

Examiner: B. Stanton

For: ISOLATION AND PRESERVATION OF FETAL AND NEONATAL HEMATOPOIETIC STEM AND PROGENITOR CELLS OF THE BLOOD

Attorney Docket No.: 6287-026

TRANSMITTAL

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Per the request of Examiner Brian Stanton, transmitted herewith is a copy of pages 1-6 of the Second Declaration of Dr. Irwin D. Bernstein, originally submitted July 20, 1994 in connection with Reexamination No. 90/003182, a copy of which was filed with the Amendment in connection with the above-identified patent application filed via Express Mail No. EM 325 959 114 US on October 30, 1996, which have been mislaid at the Patent and Trademark Office.

It is requested these pages be made of record in the file.

Respectfully submitted,

Date: January 16, 1997

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CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that this paper is being filed with the United States Patent and Trademark Office by facsimile transmission on January 16, 1997 (date) to facsimile telephone number (703) 305-7939

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#12
1/16/97

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:
Boyse et al.

U.S. Patent No.: 5,004,681

For: ISOLATION AND PRESERVATION OF FETAL AND NEONATAL HEMATOPOIETIC STEM AND PROGENITOR CELLS OF THE BLOOD

Group Art Unit: 1808

Examiner: Susan M. Dadio

Reexam. No. 90/003182

Reexam. Request Filed: August 30, 1993

Attorney Docket No.: 6287-021

SECOND DECLARATION OF DR. IRWIN D. BERNSTEIN

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

SIR:

I, DR. IRWIN D. BERNSTEIN, do declare and state that:

1. I am a citizen of the United States residing at 4949 Stanford Avenue Northeast, Seattle, Washington 98105.

2. I received the degree of Medical Doctor from New York University, New York, New York in 1967. I received the degree of Bachelor of Sciences in Biology from Trinity College, Hartford, Connecticut in 1963.

3. I presently hold the positions of Professor of Pediatrics, and Director of Division of Pediatric Hematology of the University of Washington School of Medicine,

Seattle, Washington; and Member and Program Head of Pediatric Oncology, Fred Hutchinson Cancer Research Center, Seattle, Washington.

4. My complete academic background, publications, professional experience and honors are set forth in my *curriculum vitae*, a copy of which is annexed as Exhibit A to the Declaration of Irwin D. Bernstein which was filed on February 4, 1994 for the above-identified reexamination (hereinafter the "first Bernstein Declaration").

5. My present employment involves both research and clinical practice. My research focuses on hematopoietic stem cells (both normal and malignant), and antibody-targeted therapy for hematopoietic malignancies. I am presently conducting and have in the past conducted research directed to the isolation and characterization of bone marrow cells required for hematopoietic reconstitution in humans. I routinely interact in my research activities with the bone marrow transplant group at the Fred Hutchinson Cancer Research Center. I have been conducting research directly relating to hematopoietic cells since approximately 1979. My clinical practice is mainly general pediatric oncology, but I also have been responsible for some patients undergoing bone marrow transplantation and some patients undergoing transfusion with autologous peripheral blood mononuclear cells. I am the author or co-author of over 190 research, technical or proprietary publications of which more than 50 pertain to hematopoietic reconstitution, hematopoiesis or hematopoietic stem or progenitor cells. In particular, I have authored or co-authored approximately eighteen publications directly pertaining to hematopoietic reconstitution.

6. I have read and am familiar with United States Patent No. 5,004,681 ("the '681 Patent"). I have read and am familiar with the Request for Reexamination of the '681 Patent filed by Cryo-Cell International, Inc., the Order

Granting Request for Reexamination dated November 5, 1993, Patent Owner's Statement Under 37 C.F.R. § 1.530 filed February 4, 1994, Requester's Reply to Patent Owner's Statement dated March 17, 1994, and the Office Action in Reexamination dated May 20, 1994. I have read and am familiar with the following 12 references, relied upon by the Examiner in the Office Action dated May 20, 1994:

Shope et al., Proceedings of the Society for Experimental Biology and Medicine, 157:326-329 (1978) ("Shope et al.")

Moretti et al., Fetal Liver Transplantation, pp. 121-133, copyright 1985 by Alan R. Liss, Inc., Prog. Clin. Biol. Res. 193:121-133 ("Moretti et al.")

Fliedner and Calvo, Fetal Liver Transplantation, Current Concepts and Future Directions, "Proceedings of the First International Symposium on Fetal Liver Transplantation, Pesaro, Italy, September, 1979," pp. 305-309 ("Fliedner et al.")

Ende, Virginia Medical Monthly, 99:276, March, 1972 ("Ende")

Löwenberg, Bob, Fetal Liver Cell Transplantation, Role and nature of the fetal haemopoietic stem cell, Ultgeverij Waltman-Delft, 1975, Section 1.3.6, "Fetal liver cell transplantation in man," pp. 25-28 and p. 36 ("Löwenberg")

Ueno et al., Exp. Hematol. 9:716-722 (August 1981) ("Ueno et al.")

Prindull et al., Acta Paediatr. Scand., 67:413 (1978) ("Prindull et al.")

Korbling et al., Blood 67(2):529-532 (1986) ("Korbling et al.")

Valeri, in *Blood Banking and the Use of Frozen Blood Products*, Ch. 1, CRC Press, pp. 1-7 (1976) ("Valeri")

Karp et al., Am. J. Hematol. 18:243-249 (1985) ("Karp et al.")

Gorin, Clin. Haematol. 15:19-48 (1986) ("Gorin")

Nakahata and Ogawa, J. Clin. Invest. 70:1324-1328 (1982) ("Nakahata et al.")

7. I have read claims 1-9 of the '681 Patent. The invention described and claimed in the '681 Patent is a composition of viable human neonatal or fetal

hematopoietic stem cells derived from the blood in combination with cryopreservative. In some claims, the composition also contains, in addition to such cryopreserved stem cells, viable human neonatal or fetal hematopoietic progenitor cells, whole neonatal or fetal blood, or anticoagulant. In other claims, the cryopreservative is specified, or the stem or progenitor cell is characterized by certain abilities.

8. I make this Declaration to supplement the remarks I made in the first Bernstein Declaration, to explain further the basis for my conclusion that the claimed invention of the '681 Patent was not obvious to one of ordinary skill in the art at about the filing date of the application leading to the '681 Patent (November 12, 1987).

9. Hematopoietic stem and progenitor cells are the cells from which the mature functional cells circulating in the blood derive. Stem cells are the most primitive cells in the hematopoietic lineage; they have extensive proliferative capacity and the ability to generate other stem cells as well as to differentiate into the progenitor cells, which in turn can differentiate into the mature cells. The mature cells include erythrocytes (red blood cells), granulocytes, monocytes/macrophages, megakaryocytes, T cells, B cells, and non-T, non-B cells. In progressing through the hierarchy of hematopoietic cells from stem cells to progenitor cells to mature cells, the cells have progressively more restricted differentiation capacity, *i.e.*, less ability to produce mature cells of different types within the different blood cell lineages. The blood cell lineages consist of both the myeloid (including the mature cells that are erythroid cells (*e.g.*, red blood cells), granulocytes, monocytes/macrophages, and megakaryocytes) and lymphoid (including the mature cells that are T-, B-, and non-T, non-B cells) lineages. Stem cells are pluripotent in that they have the greatest potential, by differentiation, to produce the various cells of the different blood cell lineages. Progenitor cells have more limited

multipotentiality and a lesser degree of proliferative capacity. The direct precursors to mature cells are unipotent, in that they can only produce their own kind. The most immature human hematopoietic stem cell is the cell with long-term, marrow repopulating ability that is able to effect hematopoietic reconstitution. I use the term "hematopoietic reconstitution," consistent with its usage in the '681 Patent, to mean "long-term," complete (multilineage) hematopoietic repopulation *in vivo*. It is the stem cell with long-term marrow repopulating ability that, in sufficient amounts, has utility for hematopoietic reconstitution. Cells other than the long-term marrow repopulating stem cell also have been termed stem cells, such as those detected by the CFU-s (spleen colony forming) assay[1] or the ability to give rise to *in vitro* blast cell colonies that can be replated *in vitro* to form secondary colonies containing the different mature blood cell types (such as disclosed by Nakahata et al.). Despite this use of the "stem cell" terminology, it was recognized in the art at about the time the application leading to the '681 Patent was filed, and is presently recognized, that the cells detected by the CFU-s assay and the cells giving rise to *in vitro* blast cell colonies had an unknown relationship to the stem cells with the ability to effect hematopoietic reconstitution. Assays which detect cells that form colonies *in vitro* of mature blood cells are detecting progenitor cells. As set forth in the Definitions section of the '681 Patent,[2] progenitor cells detected by the ability to form

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1 The CFU-s assay detects murine, not human, cells; it is a colony forming assay done *in vivo* in mice.

2 See col. 8, line 46 to col. 9, line 6.

colonies *in vitro* of mature blood cells include the BFU-E,[3] CFU-GEMM,[4] and CFU-GM.[5] The more different types of mature cells that are produced in a single colony, the broader the potentiality (breadth of differentiation ability) of the colony-forming cells thus detected. Thus, for example, CFU-GEMM are multipotential progenitor cells that are more primitive (earlier in the differentiation hierarchy) than CFU-GM. Since the definitions of hematopoietic stem and progenitor cells are operational, their presence can be determined only by the appropriate functional assay. In particular, the presence of a long-term marrow repopulating stem cell in any particular composition is only reasonably expected if that composition has been shown capable of providing long-term, complete (multilineage) repopulation of the blood components *in vivo* (*i.e.*, hematopoietic reconstitution).

10. Prindull et al. discloses cells which give rise to colonies *in vitro* composed of one type of mature cell, a myelocyte/metamyelocyte. Ueno et al. discloses (i) cells which give rise to colonies *in vitro* composed of one type of mature cell, a granulocyte, (ii) cells which give rise to colonies *in vitro* composed of monocytes/macrophages (a monocyte is the direct precursor to the macrophage, a mature cell type), and (iii) only a few cells giving rise to colonies of both granulocytes and monocytes/macrophages (*i.e.*, CFU-GM) (p. 719, Table 1 and col. 1). It is clear in view

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[3] "BFU-E = burst-forming unit-erythroid. An hematopoietic progenitor cell which is capable of producing a colony of erythroid progeny cells in semi-solid medium" (the '681 Patent at col. 8, lines 49-52).

[4] "CFU-GEMM = colony-forming unit-granulocyte, erythrocyte, monocyte/macrophage, megakaryocyte. A multipotential hematopoietic progenitor cell which is capable of producing a colony composed of granulocyte, erythrocyte, monocyte/macrophage, megakaryocyte progeny, in semi-solid medium" (the '681 Patent at col. 8, line 65 to col. 9, line 2).

[5] "CFU-GM = colony-forming unit-granulocyte, macrophage. An hematopoietic progenitor cell which is capable of producing a colony composed of granulocyte and macrophage progeny in semi-solid medium" ('681 Patent at col. 9, lines 3-6).